Testa CHALLENGE 2024

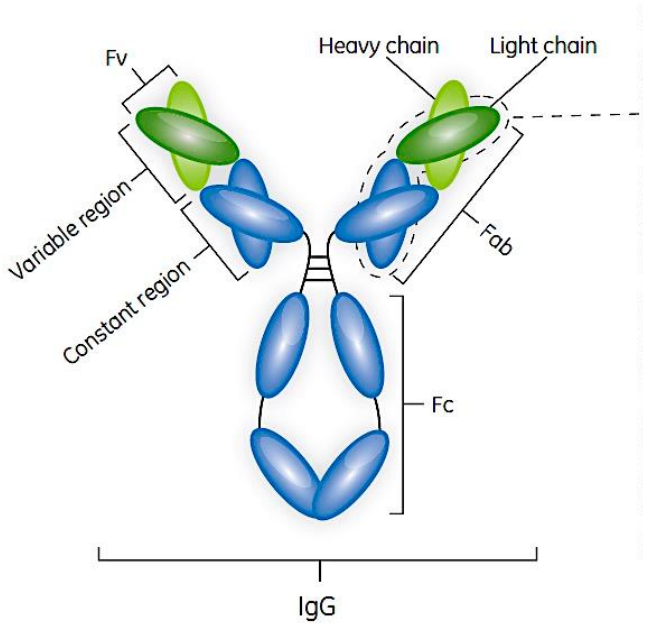
cytiva KNIGHTEC GYROS PROTEIN Technologies RI.SE Region Uppsala TestaCenter In collaboration with Cytiva

Technical description

A start to finish process – Producing and purifying a monoclonal antibody (mAb) as intermediate for ADC application.

Process outline:

- Production of mAb with CHO cells using fed-batch technique in single-use bioreactor
- Clarification of the culture using depth filtration
- Purification of mAb using affinity chromatography followed by additional polishing step with multimodal chromatography resin



Process overview

UPSTREAM

Seed culture in Wave

Main culture in stirred bag SUB

MIDSTREAM

Clarification of culture by depth filtration

Sterile filtration to bag

DOWNSTREAM

Capture on affinity column

Polishing on IEX column

ANALYTICS

In-process analytics - both USP & DSP

Analysis of final product (mAb)

Timeline

UPSTREAM		MIDSTREAM	DOWNSTREAM		ANALYTICS
Preparation of solutions & sensors/equipment Seed culture in Wave 25 bioreactor	Fed-batch cultivation in XDR-50 bioreactor including daily sampling/analytics	Clarification of culture Sterile filtration of clarified material Product conc. analysis	Affinity capture of mAb	Polishing on IEX	In-process (product yield) Quality of final product (SEC, PAGE, peptide mapping)
W0&1. 23/8-28/8	W1&2. 28/8-6/9	W2. 6/9	W3. 9/9	W3. 10-11/9	W2&3. 6/9-13/9

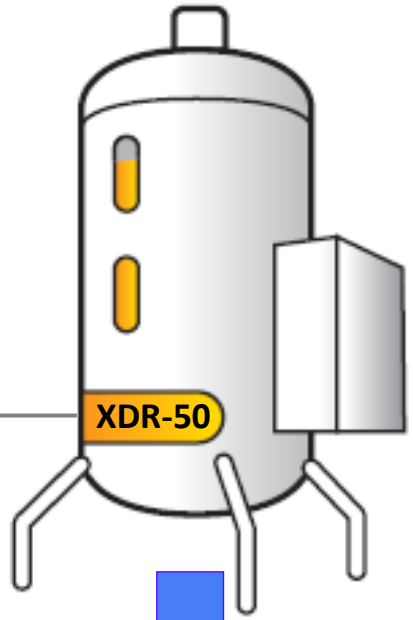
Upstream process

1. Reactor preparation
Installation of reactor bag, insertion of sterile probes. Preparation of feed solutions

2. Seed preparation
Starting from a cryovial CHO cells will be expanded in shake flasks followed by Wave 25 bioreactor cultivation to generate seed culture for the production bioreactor

3. Sterile filtration of culture medium
28 L of culture medium pumped through 0.2 µm NF capsule filter into the Bioreactor.

Seed culture



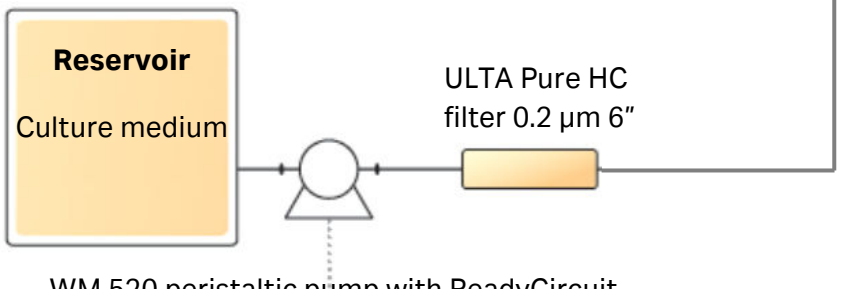
4. Inoculation of Bioreactor
≈8L seed suspension from Wave 25 bioreactor is inoculated into 28 L culture medium in XDR-50 bioreactor via a transfer line welded to appropriate tubes at the two bioreactors.

5. Fed-batch culture in Bioreactor
Cultivation performed approx. 10 days with daily addition of feed solutions and regular analysis of critical process parameters.

6. Aseptic transfer to harvest line
Aseptic transfer of cells from bioreactor via 3/8" x 5/8" C-flex drain tube connected via ReadyMate to ReadyCircuit harvest line.

Harvest using depth filtration

Affinity Chromatography using MabSelect PrismA resin



Clarification process

Harvest using depth filters

Material to process:
- 30 L *CHO* culture
- Conc.: ~3 g mAb/L

Depth filters:
- Pall Stax PDP8 & PDE2 single-use discs

Peristaltic pump

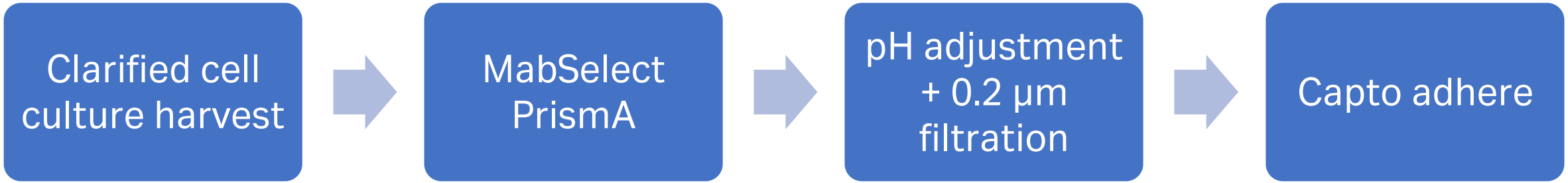
Depth filter discs

Storage bag

Clarification conditions:
- Filters flushed with purified water followed by conditioning with phosphate buffer
- Process runs in constant flow mode

Clarified solution
- Sterile filter to a 30 L bag via Pall Supor EKV capsule

Downstream process flow chart



Downstream process: Step 1

Capture step using Affinity Chromatography: MabSelect PrismA

Chromatography step 1:
- MabSelect PrismA chromatography resin
- Binding capacity: 48 g/L resin

(6 min residence time, 80% load)
- 41 g mAb is processed in 2 runs with a 427 mL column

ÄKTA pilot 600

AxiChrom 70/300

AxiChrom 70/300:
- 11.1 cm bed height
- 427 mL MabSelect PrismA

Chromatographic method:

Step	Solution	CV	Comment
Eq.	20 mM phosphate, 0.15 M NaCl, pH 7.0	3	
Sample appl.	mAb sample	7 L	6 min res.time
Wash 1	20 mM phosphate, 0.15 M NaCl, pH 7.0	5	
Wash 2	20 mM phosphate, 0.5 M NaCl, pH 7.0	1	
Elution	50 mM acetate, pH 3.5	3	Peak fractionation
Strip	100 mM acetic acid, pH 2.9	2	
CIP	0.5 M NaOH	2	15 min contact time
Re-eq.	20 mM phosphate, 0.15 M NaCl, pH 7.0	5	

Total run time: ~240 min.

Eluate:
- Collect eluates in Duran bottles
- pH adjust pooled eluates with 2 M Tris base to pH 5.

Downstream process: Step 2

Polishing step using multimodal chromatography: Capto adhere resin

Capto adhere can remove key contaminants such as:

- host cell DNA (hcDNA)
- host cell proteins (HCP)
- leached protein A
- mAb dimers and larger aggregates
- viruses

Chromatography:

- Capto adhere resin
- Flow-through mode
- Sample load: 250 g mAb/L resin
- 0.22 µm filtered prior to chromatography run
- 1 run with a 160 mL column

ÄKTA pure 150

Chromatographic method:

Step	Solution	CV	Comment
Eq.	100 mM acetate, 0.13 M NaCl, pH 5.0	7	4 min res.time
Sample appl.	mAb sample	2.5 L	
Wash *	100 mM acetate, 0.13 M NaCl, pH 5.0	7	
Rinse	water	3	8 min res.time
CIP	1 M NaOH	3	
Re-eq.	100 mM acetate, 0.13 M NaCl, pH 5.0	10	4 min res.time

* Fraction collection to be stopped at 100 mAU and the process is continued with rinse.

Total run time: ~210 min.

AxiChrom 50/300:

- 8 cm bed height
- 160 mL Capto adhere

Eluate:

- Sterile filtered into 1L flasks

Analytics

Upstream Process Analytics

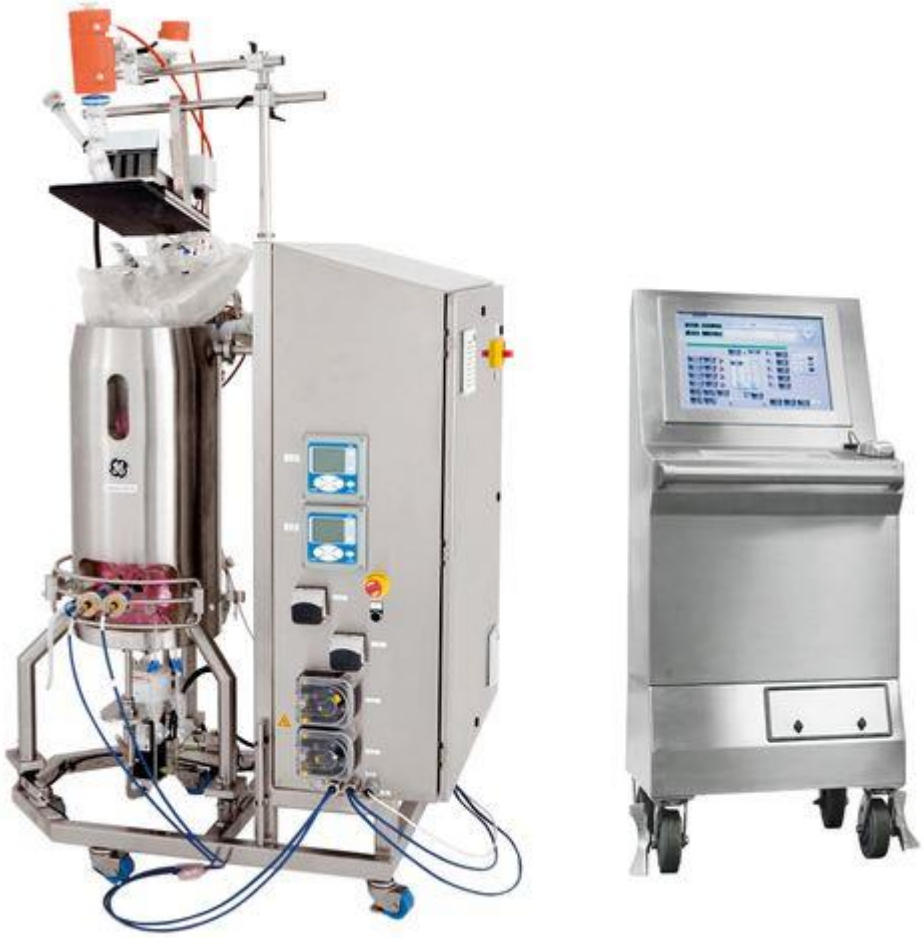
- Cell concentration and viability (Vi-Cell XR)
- Nutrients and metabolites concentration (Cedex Bio)
- Culture osmolarity (Osmo1)

Product Yield and Purity

- mAb concentration on protein A column
- Aggregates and fragments (SEC column)
- mAb size and impurity profile (PAGE)
- mAb identity - peptide mapping (Reversed-Phase chromatography)
- Host cell protein content (Gyrolab)

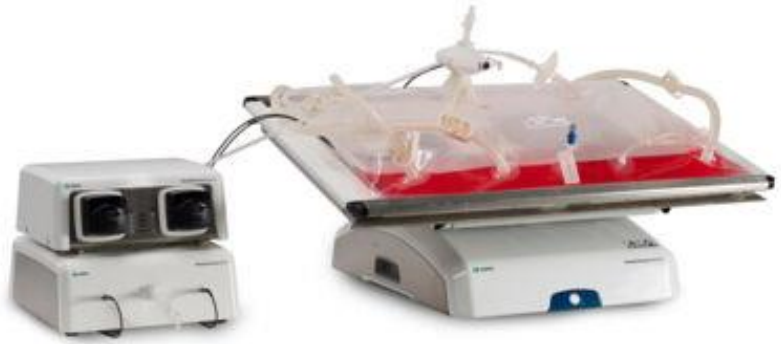
Instruments and consumables

Single-use bioreactors



XDR-50 bioreactor

https://www.cytivalifesciences.com/en/us/shop/cell-culture-and-fermentation/single-use-bioreactor-systems/xcellerex-xdr-50-to-2000-single-use-stirred-tank-bioreactors-p-06177



Wave rocking bioreactor

https://www.cytivalifesciences.com/en/se/shop/cell-culture-and-fermentation

Stax™ Disposable Depth Filter Systems

https://shop.pall.com/us/en/biotech/depth-filtration/zidgri78m6c

Chromatography systems and columns

ÄKTA pilot 600

https://www.cytivalifesciences.com/en/us/solutions/bioprocessing/products-and-solutions/downstream-bioprocessing/akta-pilot-600

ÄKTA pure 150

https://www.cytivalifesciences.com/en/us/shop/chromatography/chromatography-systems/akta-pure-p-05844

AxiChrom 50/300

https://www.cytivalifesciences.com/en/us/shop/chromatography/columns/process-columns/axichrom-50-to-200-mm-chromatography-columns-p-06215

AxiChrom 70/300